

Response to Notice of Non-Compliant Amendment

Page 2 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)Amendments to the Specification

Please Amend the title as follows:

DRUG DISCOVERY METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH

Please replace the paragraph beginning at page 2, line 24, with the following amended paragraph.

The present invention is directed to a collection of polypeptides that includes at least two polypeptides. Each polypeptide includes a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 ~~SEQ ID NO:1~~ beginning at any amino acid from about 119 to about 124 and ending at any amino acid from about 258 to about 275. At least two consecutive amino acids within the regions of amino acids 129-137, or amino acids 182-189, or amino acids 257-264 ~~as depicted at SEQ ID NO:1~~ are replaced by an amino acid sequence that includes Xaa<sub>n</sub>, wherein n is from about 5 to about 21, and each Xaa is independently a random amino acid. Two examples of polypeptides that are members of one collection are SEQ ID NO:33 and SEQ ID NO:34. Each member of the collection may further include a cell-permeant region fused to the amino terminal end of the polypeptide. Preferably, the cell-permeant region includes an amino acid sequence YGRKKRRQRRR (SEQ ID NO:2), RQIKIWFQNRRMKWKK (SEQ ID NO:3), RQIKIWFPNRRMKWKK (SEQ ID NO:4), or RQPKIWFPNRRPKWKK (SEQ ID NO:5). The invention is further directed to a cell that includes a member of the collection of polypeptides, and a population of cells that includes two or more cells, wherein each member of the population includes one polypeptide of the collection of polypeptides.

Please replace the paragraph beginning at page 3, line 11, with the following amended paragraph.

The invention also provides a polypeptide selected from the group consisting of an amino acid sequence SEQ ID NO:2 fused to an amino terminal end of a fragment of the carboxy

**Response to Notice of Non-Compliant Amendment**

Page 3 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 SEQ ID NO:1 beginning at any amino acid from about 119 to about 124 and ending at any amino acid from about 258 to about 275, wherein at least two consecutive amino acids within the regions of amino acids 129-137, or amino acids 182-189, or amino acids 257-264 as depicted at SEQ ID NO:1 are replaced by an amino acid sequence including Xaa<sub>n</sub>, wherein n is from about 5 to about 21, and each Xaa is independently a random amino acid. The polypeptide may further include a cell-permeant region fused to the amino terminal end of the polypeptide. The invention is further directed to a cell that includes a member of the collection of polypeptides.

Please replace the paragraph beginning at page 3, line 22, with the following amended paragraph.

Further provided by the invention is a collection of polynucleotides including at least two polynucleotides. Each polynucleotide includes a coding sequence encoding a polypeptide that includes a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 SEQ ID NO:1 beginning at any amino acid from about 119 to about 124 and ending at any amino acid from about 262 to about 275, wherein at least two consecutive amino acids within the regions of amino acids 129-137, or amino acids 182-189, or amino acids 257-264 as depicted at SEQ ID NO:1 are replaced by an amino acid sequence including Xaa<sub>n</sub>, wherein n is from about 5 to about 21, and each Xaa is independently a random amino acid. The polypeptide may further include a cell-permeant region fused to the amino terminal end of the polypeptide. The nucleotide sequence of the coding sequence encoding the Xaa<sub>n</sub> may consists of a nucleotide sequence NNK<sub>o</sub>, wherein N is independently a random nucleotide, K is independently a guanine or a thymine, and wherein o is from about 5 to about 21. The polynucleotide may be present in a vector, for instance, a retrovirus vector. The invention is further directed to a cell that includes a member of the collection of polynucleotides, and a population of cells that includes two or more cells, wherein each member of the population includes one polynucleotide of the collection of polynucleotides.

**Response to Notice of Non-Compliant Amendment**

Page 4 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

Please replace the paragraph beginning at page 4, line 28, with the following amended paragraph.

Also provided by the invention is a method for identifying a polypeptide within a collection that prevents cell death after exposure to a pathogen or a toxin. The method includes providing a cell that contains a polypeptide that is a member of a collection of polypeptides including at least two polypeptides. Each polypeptide includes a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 SEQ ID NO:1 beginning at any amino acid from about 119 to about 124 and ending at any amino acid from about 258 to about 275, wherein at least two consecutive amino acids within the regions of amino acids 129-137, or amino acids 182-189, or amino acids 257-264 as depicted at ~~SEQ ID NO:1~~ are replaced by an amino acid sequence including Xaa<sub>n</sub>, wherein n is from about 5 to about 21, and each Xaa is independently a random amino acid. ~~[[.]]~~ The cell is exposed to a pathogen or a toxin, and whether the polypeptide prevents cell death is determined by incubating the cell under conditions such that the pathogen or the toxin kills a cell that does not include a polypeptide that prevents cell death after exposure to a pathogen or a toxin. The presence of a cell that proliferates indicates the polypeptide prevents cell death after exposure to a pathogen or a toxin. The pathogen may be, for instance, a virus or a microbe. Examples of microbes include a bacterium, a rickettsia, and a fungus. Examples of toxins include a biological toxin or a chemical toxin.

Please replace the paragraph beginning at page 5, line 16, with the following amended paragraph.

The invention provides a method for identifying a polypeptide within a collection that binds a pathogen, a toxin, a polypeptide, or a polynucleotide. The method includes providing a cell that includes a polypeptide that is a member of a collection of polypeptides including at least two polypeptides. Each polypeptide includes a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 SEQ ID NO:1 beginning at any amino acid from about 119 to about 124 and ending at any amino acid from

**Response to Notice of Non-Compliant Amendment**

Page 5 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

about 258 to about 275, wherein at least two consecutive amino acids within the regions of amino acids 129-137, or amino acids 182-189, or amino acids 257-264 as depicted at SEQ ID NO:1 are replaced by an amino acid sequence including Xaa<sub>n</sub>, wherein n is from about 5 to about 21, and each Xaa is independently a random amino acid. The cell is exposed to a pathogen or a toxin, and whether the polypeptide prevents cell death is determined by incubating the cell under conditions such that the pathogen or the toxin kills a cell that does not include a polypeptide that prevents cell death after exposure to a pathogen or a toxin. The presence of a cell that proliferates indicates the polypeptide binds the pathogen, the toxin, a polypeptide, or a polynucleotide. The pathogen may be, for instance, a virus or a microbe. Examples of microbes include a bacterium, a rickettsia, and a fungus. Examples of toxins include a biological toxin or a chemical toxin.

Please replace the paragraph beginning at page 6, line 10, with the following amended paragraph.

Figure 2. Figure 2A, Specific examples of members (Adaptein-1 and Adaptein-2) of a collection of polypeptides of the present invention. Figure 2B, an alignment of the Adaptein nucleotide sequences with the CCD nucleotide sequence. A-1, Adaptein-1; A-2, Adaptein-2; CCD, amino acids 119-275 of VEE virus capsid polypeptide carboxy terminal portion; HindIII and XhoI, restriction endonuclease sites; dashes indicate an absence of a nucleotide. Figure 2C, an alignment of the Adaptein amino acid sequences with the CCD amino acid sequence. Dashes indicate an absence of an amino acid.

Please replace the paragraph beginning at page 10, line 22, with the following amended paragraph.

The present invention also includes a population of cultured cells including two or more cells, where each cell of the population includes one polypeptide of one collection of polypeptides. The present invention also provides individual cultured cells containing a polypeptide that is a member of a collection of polypeptides. The cells may be prokaryotic or

## Response to Notice of Non-Compliant Amendment

Page 6 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

eukaryotic, preferably, eukaryotic, more preferably, vertebrate, most preferably, mammalian. Examples of useful mammalian cultured cells include 293 cells, macrophage cells, including J774 and RAW 264.7, HeLa cells, and Vero cells, each of which is available from the ATCC.

Please replace the paragraph beginning at page 7, line 17, with the following amended paragraph.

Each polypeptide of a collection includes a fragment of an amino acid sequence having a peptide backbone conformation that acts to display a variable amino acid sequence on the surface of the polypeptide. Variable amino acid sequences are described in greater detail herein. A fragment of an amino acid sequence having this peptide backbone conformation is also referred to herein as a carrier polypeptide or scaffold polypeptide. A preferred example of such an amino acid sequence is a carboxy terminal portion of the Venezuelan equine encephalitis (VEE) virus capsid polypeptide. An example of the amino acid sequence of the VEE capsid polypeptide is available at GenBank Accession Number L01443. A preferred carboxy terminal portion of the VEE capsid polypeptide, also referred to herein as "CCD," begins at about amino acid 119 and ends at about amino acid 275, and correlates with amino acids 1-157 ~~[[is]]~~ depicted at SEQ ID NO:1 (see Figure 1). Amino acids 119-275 of the CCD are encoded by nucleotides 7916-8386 of GenBank Accession Number L01443, and are shown in Figure 1. This amino acid sequence forms a trypsin resistant and chymotrypsin-resistant structure of predominantly  $\beta$ -sheets, with small loops connecting sequential strands.

Please replace the paragraph beginning at page 8, line 3, with the following amended paragraph.

Accordingly, in one aspect of the invention, a collection of polypeptides includes at least two polypeptides, where each polypeptide includes a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 ~~SEQ ID NO:1~~. The fragment begins at any amino acid from about 119 to about 124, preferably, about 119. The fragment ends at any amino acid from about 258 to about 275, preferably, about 275. The

## Response to Notice of Non-Compliant Amendment

Page 7 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

fragment further includes a variable amino acid sequence, which is described in detail herein. The variable amino acid sequence replaces from about 1 to about 4, more preferably, from about 2 to about 3, most preferably, about 2, amino acids within 1 of 3 regions of the fragment. The 3 regions are amino acids 129-137, amino acids 182-189, and amino acids 257-264 of SEQ ID NO:1. Preferably, a variable amino acid sequence replaces amino acids within the third region, i.e., amino acids 257-264. Preferably, the amino acids within the third region that are replaced by the variable amino acid sequence are amino acids 260-261 of SEQ ID NO:1.

Please replace the paragraph beginning at page 8, line 26, with the following amended paragraph.

The variable amino acid sequence that is used to replace amino acids of a fragment of the carboxy terminal portion of the VEE capsid polypeptide available at GenBank Accession Number L01443 SEQ ID NO:1 has the amino acid sequence Xaa<sub>n</sub>. The "n" can be, in increasing order of preference, from about 5 to about 21, from about 6 to about 18, from about 6 to about 12, most preferably, about 6. Each Xaa is independently any amino acid, preferably one of the 20 natural amino acids. Thus, in a single collection of polypeptides, each member of the collection has a variable amino acid sequence that has the same number of amino acids (i.e., about 5 to about 21) but a different amino acid sequence. Accordingly, in the aspect of the present invention where amino acids 260-261 are replaced by Xaa<sub>n</sub> and n is 6, a polypeptide of a collection of polypeptides has the following amino acid sequence: amino acids 119-259 of the VEE capsid polypeptide available at GenBank Accession Number L01443 as depicted at SEQ ID NO:1 followed by the amino acid sequence XaaXaaXaaXaaXaaXaa (SEQ ID NO:11) followed by amino acids 262-275 of the VEE capsid polypeptide available at GenBank Accession Number L01443 as depicted at SEQ ID NO:1.

**Response to Notice of Non-Compliant Amendment**

Page 8 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

Please replace the paragraph beginning at page 16, line 4, with the following amended paragraph.

The present invention is further directed to methods for identifying a polypeptide within a collection. In one aspect, the method identifies a polypeptide within a collection, where the polypeptide prevents cell death after the cell is exposed to a pathogen or a toxin. In another aspect, the method identifies a polypeptide within a cell, where the polypeptide binds a pathogen, a toxin, a polypeptide, [[of]] or a polynucleotide. The methods include providing a cell that contains a polypeptide of the present invention, exposing the cell to a pathogen or a toxin, and determining if the polypeptide of the present invention prevents cell death. To determine if the polypeptide prevents cell death, the cell is incubated under conditions that result in the pathogen or the toxin killing those cells that do not contain a polypeptide of the present invention, or contain a polypeptide of the present invention that does not protect the cell. The presence of a living cell indicates the polypeptide prevents cell death after exposure to a pathogen or a toxin. Without intending to be limited by theory, it is expected that a variable amino acid sequence of the polypeptides of the present invention will protect a cell from the pathogen or toxin by binding to target polypeptides or nucleotides of either viral or host cell origin. For instance, an amino acid sequence may bind to a pathogen polypeptide or nucleic acid sequence and prevent replication. A variable amino acid sequence may interact with host cell polypeptides or nucleic acids to protect the cell from pathogen challenge. For example, the variable amino acid sequence may selectively inhibit a cellular protease required for viral protein processing, or may down-regulate the expression and/or transport of host cell receptors required for pathogen or toxin entry.

Please replace the paragraph beginning at page 19, line 14, with the following amended paragraph.

In one aspect of the invention, the polypeptide of the present invention can be expressed in a cell that is to be exposed to a pathogen or a toxin. Typically, a polynucleotide encoding the polypeptide will be present in a cell in a vector as described herein, preferably

**Response to Notice of Non-Compliant Amendment**

Page 9 of 10

Applicant(s): Watowich et al.

Serial No.: 09/981,286

Filed: October 15, 2001

For: METHODS FOR IDENTIFYING POLYPEPTIDES THAT PREVENT CELL DEATH (As Amended)

inserted into the chromosome. In another aspect, the polypeptide of the present invention can be introduced to the cell. Typically, such a polypeptide will contain a cell-permeant region that will allow the polypeptide to traverse the cell membrane. In this aspect, the polypeptide can be introduced to the cell before, at the same time, or after exposing the cell to the pathogen or toxin.